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### **REMARKS**

Claims 1 to 9 and 21 to 27 are pending and are variously rejected under 35 U.S.C. §§ 102 and 112, second paragraph. Previously withdrawn claims 10 to 20 have been canceled by amendment herein, without prejudice or disclaimer. Claim 22 has been amended as shown above. No new matter has been added by way of these amendments and the entry thereof is respectfully requested.

Applicants acknowledge with appreciation that the rejections based on 35 U.S.C. § 112, written description and § 103 have been withdrawn.

#### **Obviousness-Type Double Patenting**

Claims 1-9 and 21-27 are rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-8 and 16-18 of U.S. Patent No. 6,638,752. (Office Action, page 3). Applicants submit the appropriate terminal disclaimer herewith.

#### **Rejection Under 35 U.S.C. §112, First Paragraph, Written Description**

Claim 22 remains rejected under 35 U.S.C. §112, first paragraph, as allegedly not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. In particular, the Office reiterates "the instant specification satisfies the written description requirement only with regards to genetically engineered bacteria that function as biotectors. The rejected claim is drawn to bacteria that 'shelter' the biotectors, i.e., the biotectors are genetically engineered bacteria that are 'sheltered' within other bacteria. ... This embodiment, contrary to Applicant's assertion, does not meet the written description requirement." (Office Action, page 4).

Applicants traverse the rejection for the reasons of record. *See*, Response filed November 6, 2000. In particular, it is reiterated that the application as filed does in fact contemplate and describe living biotectors "sheltered" within other living entities. Nonetheless, solely to advance prosecution, claim 22 has been amended as shown above, and, accordingly, this rejection has been obviated.

#### **Rejection Under 35 U.S.C. §102(b)**

All examined claims are rejected under 35 U.S.C. §102(b) as allegedly obvious over Karube & Nakanishi (1994) *Current Opin Biotech* 5:54-59 (hereinafter "Karube") in light of

Sleight. (Office Action, page 5). It is maintained that Karube teaches cells comprising biodetectors for the detection and analysis of specific substrates. In addition, Sleight is cited for allegedly teaching the processes and components involved in signal transduction and illustrates that the biodetector disclosed by Karube inherently possesses the properties of the claimed biodetectors. (Office Action, page 5).

Applicants traverse the rejection and supporting remarks.

In order to be an anticipatory reference, the reference cited by the Office must disclose each and every element of the claims, including each and every functional or biological limitation. *See, e.g., Hybritech v. Monoclonal Antibodies*, 231 USPQ 81 (Fed. Cir. 1986); M.P.E.P § 2173.05(g) Functional Limitations, Eighth Edition. Moreover, the single source must disclose all of the claimed elements arranged as in the claims. *See, e.g., Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913 (Fed. Cir. 1989). Simply put, the law requires identity as between the prior art disclosure and the invention. *See, e.g., Kalman v. Kimberly-Clark Corp.* 218 USPQ 781 (Fed. Cir. 1983), *cert. denied*, 484 US 1007 (1988). Further, to support an anticipation rejection based on inherency, the Office must provide factual and technical grounds establishing that the inherent feature necessarily flows from the teachings of the reference. *See, e.g., Ex parte Levy*, 17 USPQ2d 1461, 1464 (BPAI 1990). Inherency cannot be established by probabilities or possibilities. *See, e.g., Continental Ca Co. USA, Inc. v. Monsanto Co.* 20 USPQ2d 1746, 1749 (Fed. Cir. 1987). Thus, the references must teach all elements of the claims, explicitly or inherently, including functional limitations such as biological function.

The Examiner has repeatedly concluded that Karube in light of Sleight teaches the existence of biodetectors as claimed. In particular, the Examiner maintains that the fact that the Karube discloses genetically engineered cells that use light to detect the effect of metals and pesticides on cells creates a presumption that the cells have all the elements of Applicants' claimed biodetectors. Additionally, the Office maintains that Sleight's review of signal transduction pathways provides the elements absent for Karube.

The Examiner's conclusions are improperly based on incorrect interpretations of the claimed invention and incorrect interpretations of what the references actually teach. Moreover, these conclusions are entirely contradicted by the evidence of record. Thus, Applicants submit that the Office has not met its burden of establishing a *prima facie* case of anticipation because Karube in light of Sleight does not inherently or expressly disclose the required ligand-activated signal transduction pathway of the claimed biodetectors.

As a threshold matter, Applicants note that the claimed invention is not, as asserted by the Examiner, directed to biodetectors of any and all configuration. In fact, the claims clearly

recite that the signal-converting element of the claimed biodetectors must comprise an extracellular ligand-specific moiety that selectively recognizes a selected substance. Moreover, the signal-converting element must also include an intracellular component that is activated by the specific binding of the substance to the extracellular component. Thus, the claims are directed to biodetectors in which a ligand must specifically bind to an extracellular moiety that in turn activates the intracellular moiety of the signal-converting element. The ligand itself is not transported across the cell membrane.

In contrast to Applicants' claims, Karube does not disclose or suggest biodetectors that function via signal transduction pathways. Rather, Karube relates entirely to detection of substances that both (1) bind non-specifically to the cell and (2) actually cross the cell membrane (*e.g.*, metals and pesticides). With regard to the first point, Applicants note that it is (and was) known by persons of ordinary skill in the field of signal transduction that metals and toxins bind non-specifically to the surface of a host cell, and are taken into the cell via ion channels or other non-specific entry mechanisms. (*see, e.g.*, Monteilh-Zoller Abstract and internet printout from chembytes attached hereto). Plainly, the non-specific entry mechanisms disclosed in Karube are not the "extracellular ligand specific domain," as recited in the claims.

Moreover, Karube also fails to describe, expressly or inherently, biodetectors comprising an intracellular domain that is activated by specific binding of the ligand to the extracellular moiety. Instead, in Karube's detectors, the substance (*i.e.*, metal or pesticide) binds non-specifically and then is itself transported across the cell membrane. This is quite a different proposition from the claimed biodetectors in which the ligand binds specifically and binding triggers the activation of the intracellular domain. Simply put, Karube does not disclose, inherently or explicitly, biodetectors in which binding of a ligand activates a signal transduction pathway. Accordingly, this reference cannot anticipate the pending claims.

Furthermore, because Karube is entirely unrelated to signal transduction pathways, the disclosure cannot be properly viewed in light of Sleight, which relates entirely to signal transduction pathways, all of which are characterized by the fact that the ligand does not itself enter the cell.

Thus, it is plain that a biodetectors as claimed, are not disclosed in Karube, either alone or in light of general references regarding signal transduction pathways.

**CONCLUSION**

Applicants respectfully submit that the claims comply with the requirements of 35 U.S.C. §112 and define an invention that is patentable over the art. Accordingly, a Notice of Allowance is believed in order and is respectfully requested.


If the Examiner notes any further matters that the Examiner believes may be expedited by a telephone interview, the Examiner is requested to contact the undersigned.

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## **TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions.**

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Trace metal ions such as  $\text{Zn}(2+)$ ,  $\text{Fe}(2+)$ ,  $\text{Cu}(2+)$ ,  $\text{Mn}(2+)$ , and  $\text{Co}(2+)$  are required cofactors for many essential cellular enzymes, yet little is known about the mechanisms through which they enter into cells. We have shown previously that the widely expressed ion channel TRPM7 (LTPC7, ChaK1, TRP-PLIK) functions as a  $\text{Ca}(2+)$ - and  $\text{Mg}(2+)$ -permeable cation channel, whose activity is regulated by intracellular  $\text{Mg}(2+)$  and  $\text{Mg}(2+)$ .ATP and have designated native TRPM7-mediated currents as magnesium-nucleotide-regulated metal ion currents (MagNum). Here we report that heterologously overexpressed TRPM7 in HEK-293 cells conducts a range of essential and toxic divalent metal ions with strong preference for  $\text{Zn}(2+)$  and  $\text{Ni}(2+)$ , which both permeate TRPM7 up to four times better than  $\text{Ca}(2+)$ . Similarly, native MagNum currents are also able to support  $\text{Zn}(2+)$  entry. Furthermore, TRPM7 allows other essential metals such as  $\text{Mn}(2+)$  and  $\text{Co}(2+)$  to permeate, and permits significant entry of nonphysiologic or toxic metals such as  $\text{Cd}(2+)$ ,  $\text{Ba}(2+)$ , and  $\text{Sr}(2+)$ . Equimolar replacement studies substituting 10 mM  $\text{Ca}(2+)$  with the respective divalent ions reveal a unique permeation profile for TRPM7 with a permeability sequence of  $\text{Zn}(2+)$  approximately  $\text{Ni}(2+) \gg \text{Ba}(2+) > \text{Co}(2+) > \text{Mg}(2+) \geq \text{Mn}(2+) \geq \text{Sr}(2+) \geq \text{Cd}(2+) \geq \text{Ca}(2+)$ , while trivalent ions such as  $\text{La}(3+)$  and  $\text{Gd}(3+)$  are not measurably permeable. With the exception of  $\text{Mg}(2+)$ , which exerts strong negative feedback from the intracellular side of the pore, this sequence is faithfully maintained when isotonic solutions of these divalent cations are used. Fura-2 quenching experiments with  $\text{Mn}(2+)$ ,  $\text{Co}(2+)$ , or  $\text{Ni}(2+)$  suggest that these can be transported by TRPM7 in the presence of physiological levels of  $\text{Ca}(2+)$  and  $\text{Mg}(2+)$ , suggesting that TRPM7 represents a novel ion-channel mechanism for cellular metal ion entry into vertebrate cells.



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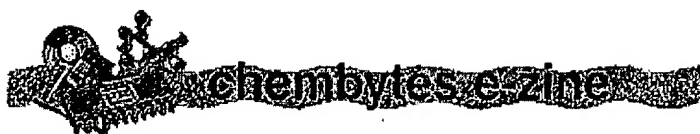
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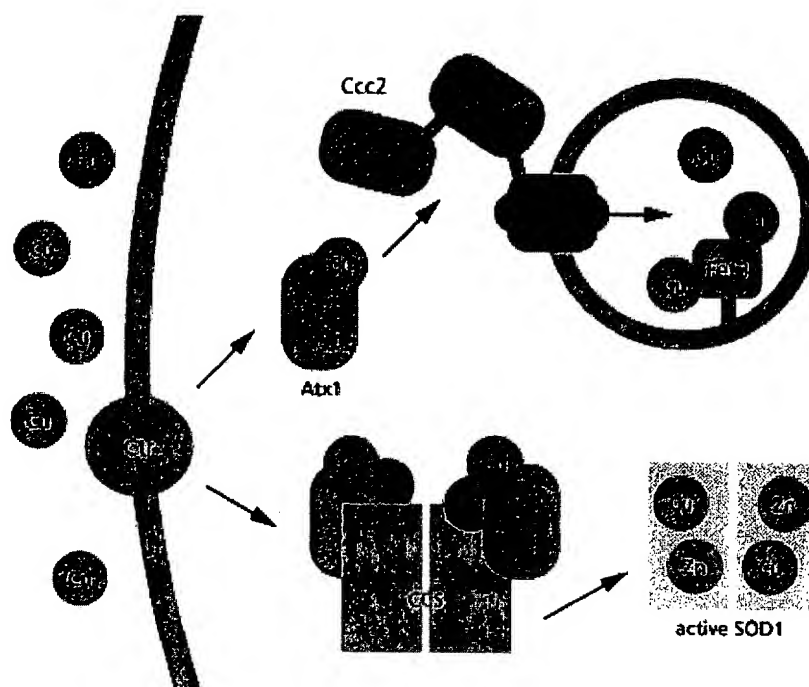
## The copper cops

**Like imprudent girls and unfolded proteins, certain metal ions in living cells may need chaperones. Michael Gross finds out why**

Copper is an essential element in all living organisms, where it serves as a cofactor in several important enzymes. On the other hand, the ease with which it embarks on both redox and coordination chemistry implies that copper ions floating freely in the cell (as is the case for the less reactive sodium and potassium ions, for instance) would be quite dangerous. Measurements confirm that the cytoplasm does not contain detectable amounts of free copper ions. Which begs the question: where do the metal enzymes get it from?

Since 1997, two pathways of intracellular copper trafficking have been described. Both involve membrane transporters and soluble transport proteins called metallochaperones, because they seem to protect their charges from inappropriate interactions. Both have been studied in yeast, and genes for homologous proteins have been identified in the human genome. One pathway supplies the cytoplasmic enzyme superoxide dismutase (SOD) with copper, while the other targets organelles such as chloroplasts and mitochondria. Let's focus on the latter one, which is backed up with more structural information.

In the cell membrane (eg of yeast), there is a copper transporter (Ctr) that controls entry of copper ions into the cell. But rather than allowing them into the cytoplasm, it hands them over to a copper chaperone named Atx1 (or Hah1 in humans). The chaperone shuttles the ion across the cytoplasm to the membrane of the target organelle, where it hands it over to a membrane transporter called Ccc2. (Two human Ccc2 homologues are known as Menkes and Wilson ATPases with reference to the diseases caused by their disruption.)



**Two metallochaperone-mediated copper delivery pathways in yeast**  
 Picture adapted with permission from *Acc. Chem. Res.*, 2001, **34**, 119  
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Atx1 must be a metal protein quite different from the average ferredoxin or zinc finger that you will find in biochemistry textbooks. Rather than binding the metal as tightly as possible, it must have finely balanced affinities to be able to take it over from one transporter, and then release it at the right moment to another one. Both transactions presumably involve protein-protein interactions of a previously unknown kind.

Some insight into how this subtle handling of metal ions might be achieved was recently gained from a series of crystal structures solved by Amy Rosenzweig's group at Northwestern University in Illinois.<sup>1</sup> First, they solved the structure of Atx1 with bound Hg(II). They found that the metal was coordinated by the sulphurs of two cysteine residues at the periphery of the globular fold (unlike catalytic copper sites which are usually buried). Then, studying the Cu(I) form of Hah1, they had a remarkable (but not unprecedented) piece of crystallographer's luck in finding the molecules in the crystal engaged in interactions that mimicked their functional interactions. The protein molecules - known to be monomeric in solution - were in fact paired up to form dimers sharing one metal ion between their juxtaposed binding sites, as if they had been caught handing it over from one protein to the other.

While Cu(I) appeared to be coordinated by two cysteines of each protein molecule, replacement by Hg(II) or Cd(II) resulted in structures with just three ligands, potentially representing a differently timed snapshot of the metal transfer process. As one of the natural target proteins of Hah1, the Menkes protein has domains that are quite similar in their fold and binding site to Hah1. This interaction observed in the crystal probably constitutes a fairly good model of what happens in the cell when the metal ion is handed



over from one to the other. Attraction between oppositely charged residues is thought to hold the complex together during the process. What is far from clear, however, is how the six separate copper binding sites of the Menkes protein relate to each other, and how the protein eventually manages to import the ion into the organelle.

This process of copper import into organelles is also extremely important for plants, which require the copper protein plastocyanin as an electron carrier in photosynthesis. Because the transporter that delivers plastocyanin into the thylakoid, the membrane enclosed compartment that contains the photosynthetic apparatus, is known not to carry folded proteins with their cofactors, there must be a separate entrance for the copper ions. Nigel Robinson's group at Newcastle University has studied this question using cyanobacteria - a group of bacteria that uses the same two-step photosynthesis system as green plants, and probably 'invented' it in the first place. Unlike plants, cyanobacteria can select which metal they will use in photosynthesis. As long as the copper supply is sufficient, they use plastocyanin like plants. But when copper runs out, they can use cytochrome c6 with a haem-iron cofactor instead.

Thus, by fiddling around with mutants and growing them under different conditions with and without copper, one can observe how they operate this switch and which genes and proteins are involved in the change. Using this approach, Robinson's group could show that two homologues of the Menkes protein in the cyanobacterium *Synechocystis* are apparently involved in transporting the copper into the thylakoid. And where do they get the copper from? Recent research from Robinson's lab suggests that bacteria, like humans, also have an Atx1 style copper chaperone, suggesting that this phenomenon is truly universal.

*Michael Gross is a science writer and consultant based at Birkbeck College, University of London. He can be contacted through his web page: [www.michaelgross.co.uk](http://www.michaelgross.co.uk)*

## References

1. A. C. Rosenzweig, *Acc. Chem. Res.*, 2001, **34**, 119.
2. S. Tottey *et al*, *J. Biol. Chem.*, 2001, **276**, 1999.

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